

THE COMPLEMENT-FIXATION TEST IN THE DIAGNOSIS OF TUBERCULOSIS, WITH A STUDY OF 135 CASES.

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THE early diagnosis of tuberculosis, or perhaps, more correctly speaking, the determination of the clinical importance of the foci which most of us are supposed to harbor, is admittedly one of the most difficult as well as most important problems in clinical medicine. So many factors have to be considered, and in certain types of cases all the available data are so indefinite, that sometimes the best we can do is to make a more or less accurate guess.

I cannot agree with those who consider the subcutaneous injection of old tuberculin, hitherto the most reliable biological test, an entirely safe procedure. If the injections are given in doses so small that no deleterious effect can be produced, we may fail in clinically active cases to get a definitely recognizable reaction, and then if repeated doses are increased very slowly, immunization may occur. This is what happens with increasing therapeutic doses. Whenever a reaction does occur there is at least a transient lowering of resistance to the infecting germ. This corresponds to Wright's "negative phase," during which phagocytic power, as shown by the opsonic index (and therefore the immunity mechanism of the body), is more or less weakened. Now, in a large proportion of suspected cases this is probably quite unimportant, because it is succeeded by a strongly positive phase. In a smaller group, however, definite harm may be produced. In a patient with a fairly low resistance, for example, and with a focus which is moderately active, the transient lowering of resistance occurring as a result of the subcutaneous test may cause increased activation of the focus, and such an event might easily turn the scale against the patient. This may not be at all obvious at the time, and, in fact, probably would not be, but might manifest itself by later clinical developments, the relationship of which to the tuberculin test might not be suspected.

I am quite well aware that many eminent clinicians, Osler among them, have declared for the harmlessness of the tuberculin test carefully employed. The biological facts above set forth are, however, indisputable, and the conclusion that positive harm might result seems unavoidable. In fact I believe I have seen such results follow its diagnostic use. Klebs¹ says that when carefully used in proper doses and in "properly selected" cases it is free from harmful effects, but adds that it should never be resorted to until

¹ Tuberculosis, 1909, p. 339.

all other means have failed. Certainly these are peculiar limitations with which to surround a perfectly safe diagnostic procedure. Lawrason Brown² in discussing the therapeutic use of tuberculin appears to offer a plausible explanation of the way in which the anaphylaxis occurs, and the same explanation would seem to apply to the harmful results which might follow its diagnostic use. He says: "The immunity mechanism seems so adjusted that whatever primary protein escapes from the focus (tubercular) is immediately disintegrated; that is, the lytic process set up by the complement is so active that anaphylatoxin is never formed and the lysis carries the splitting process on to the formation of simpler bodies. Now, when a sufficient amount, however small, of tuberculin (primary protein) is injected into the body it changes this favorable balance and the action is not carried on so far, and anaphylatoxin results. To stem these anaphylatoxins is at times difficult and their result is disastrous."

The reaction to the diagnostic dose of tuberculin given subcutaneously is undoubtedly an anaphylactic phenomenon. The patient may practically always "stem" the results, but whether he emerges from the anaphylactic storm of the tuberculin reaction with benefit or harm is difficult to determine; but if the cases are not *properly selected*, a process which may require expert judgment, the result may be "disastrous." If a strongly positive opsonic phase follows the negative phase of the reaction, a result which we are aiming at in our therapeutic use of tuberculin, which I heartily approve, then the patient may be actually benefited by the tuberculin test. This reaction corresponds to a therapeutic overdose which we consider objectionable and always try to avoid. It is only when the anaphylactic shock is too prolonged and the opsonic power of the plasma too greatly lowered that the germs may take advantage of the situation to the detriment of the patient.

Aside from the question of the possible hazards of the tuberculin test, what dependence can be placed upon it? If positive, can we confidently assume that the patient has clinical tuberculosis? If it is negative, can we exclude it? The answer to both of these questions is no. Simon³ says that from 90 to 100 per cent. of the cases of pulmonary tuberculosis give a positive reaction, while 92 per cent. of suspected cases and 56 per cent. of non-suspected cases give positive reactions. The percentage of positive reactions among cases of clinical tuberculosis is undoubtedly very large, but at least it may fail with lowered resistance, and in the very large group of cases, perhaps only moderately advanced, but with fever, either slight or severe, it certainly cannot be used. On the other hand, if 56 per cent. or even 25 per cent. of non-suspected cases give a reaction, though I am inclined to think that this is a rather

² AM. JOUR. MED. SC., 1912, cxliii, 527.

³ Infection, Immunity, etc., p. 333.

high estimate, it certainly cannot be depended upon to prove the existence of clinical tuberculosis. The work of Otis, Bandilier and Roepke⁴ seems to show that syphilis, in at least its early stages, may give rise to a positive reaction. This is especially important in view of the number of "cross-fixations" which sometimes occur in these two diseases. Independently of positive reactions in cases of syphilis, it may be fairly assumed that a large proportion of the positive reactions occurring in non-suspected cases, either in healthy individuals or those suffering from other diseases, are due to concealed foci, which may be pathologically active but clinically entirely latent. In brief, we find that the subcutaneous tuberculin test is not positive in all cases of active tuberculosis; is entirely inapplicable in the febrile state; habitually disappears in the cachectic state of late tuberculosis; and is not infrequently positive in cases in which no focus can be discovered and which are never known neither before nor after the test to show clinical manifestations of tuberculosis.

We naturally turn therefore with interest, but with not a little habitual distrust, to the complement-fixation test now on trial in the diagnosis of clinical tuberculosis, which is the main subject of this paper. This test has apparently arrived at the point where it offers much of hope without subjecting the patient to the hazards, however remote, of the subcutaneous tuberculin test. The biological basis upon which the tuberculosis complement-fixation is said to rest is substantially the same as in the case of the Wassermann test. There is, however, this difference: complement-fixation in the Wassermann test depends upon the presence of lipoid bodies, which are not at all specific for syphilis but are found in all the trypanosome diseases, and may even be produced by a meal very rich in fats, although in this latitude and with proper precautions no difficulties arise. In the tuberculosis complement-fixation test, however, the complement is said to be bound by an antibody which is absolutely specific and which is produced by the antigenic effect of the tubercle bacilli toxin. It was shown, for instance, by Citron that complement-binding antibodies were found in the blood of normal individuals after the injection of tuberculin. This is presumably the mechanism of the production of complement-binding antibodies in clinical tuberculosis by a process of auto-inoculation. Whether these antibodies will be found at any particular time or not will depend upon two factors: (1) whether toxic bacillary products are at that moment being, or have rather recently been, thrown into the blood from the tubercular foci, and (2) whether there is on the part of the organism an immunity response to the antigenic influence of these toxic bodies. Citron found that the presence of these complement-binding antibodies following the

⁴ Klebs, T. B.: p. 340.

injection of tuberculin was transitory, and this is precisely what occurs in clinical cases. I have seen a case show 50 to 75 per cent. inhibition of hemolysis in one test and none in a second test made a week later. This is not at all peculiar to tuberculosis complement-fixation. The same thing has been observed in both the Neisser and Wassermann-fixation tests. It is well known that a single negative Wassermann does not exclude syphilis, although we do not therefore reject the Wassermann test as an aid in diagnosis.

As with the Wassermann so with the tuberculosis complement-fixation test, the results appear to depend somewhat upon the antigen used. A considerable number and variety of antigens have been used during the past six years, which is the period covered by these investigations. The antigens are classified by Miller⁵ in a recent communication into four groups as follows:

Group I. Bacillary suspensions (which includes that used by Miller).

Group II. Tuberculins or filtrates (which includes Besredka's and Craig's antigens, and with which Bronfenbrenner's work has been done, as well as my own).

Group III. Bacillary extracts (used by Calmette and Masol, Much, Stimson and others).

Group IV. Tissue antigens (used by Bierbaum, Wolff, Eisner), these being analogous, of course, to alcoholic extract of the luetic liver in the Wassermann test.

Craig⁶ using a filtrate antigen, made the test upon 166 cases of pulmonary tuberculosis, together with (for control purposes) 150 syphilitics, 100 patients suffering from other diseases and 100 normal individuals. Only two of the last three groups gave a positive reaction with the test. Of the 166 cases of pulmonary tuberculosis, 142 were positive and 24 negative; 107 of these cases were clinically active, giving 103, or 95 per cent., positive. Of the 59 inactive cases, 39, or 66 per cent. were positive. He found sometimes that the same case would give positive and negative tests on different days.

Bronfenbrenner,⁷ using Besredka's antigen, studied 625 unselected hospital cases, with special reference to the association of syphilis and tuberculosis in those cases in which both of these diseases were clinically recognized or the patient gave both complement-fixation tests. Included in this 625 cases there were 174 cases of tuberculosis, 65 of which were active. Of the latter, 61, or 94 per cent. gave positive tuberculosis complement-fixation tests while 4 cases were negative. There were 27 cases of tuberculosis apparently undergoing recovery, of which 15 were positive and 12 negative.

There were 108 cases out of the 625 in which a clinical diagnosis

⁵ Jour. Lab. and Clin. Med., August, 1916, p. 816.

⁶ AM. JOUR. MED. SC., 1915, xl.

⁷ Arch. Int. Med., 1914, xiv, 787.

of syphilis was made; 41, or 38 per cent. of these gave a positive tuberculosis complement-fixation test. This is undoubtedly a very large percentage of tuberculosis among syphilitics, and fairly raised the question as to the specificity of the serum test. By a carefully conducted series of experiments, which appear to me thoroughly convincing, he has proved that the two existing positive tests when associated are due to entirely distinct and separate antibodies. He shows very clearly that the tuberculosis complement-fixation reaction is not lipotropic in character. He removes the lipoid substances from the Besredka antigen by extraction with ether without impairing its complement-binding power. He then exhausts the tubercular antibodies by repeated fixations with Besredka's antigen, leaving the Wassermann reaction unimpaired, and then, of course, in another specimen of serum exhausts the syphilitic antibodies by suitable antigens, leaving the tuberculosis complement-fixation test unimpaired. His conclusion that the tuberculosis complement-fixation test is entirely specific, and that the individual giving positive fixation of complement with both this and the Wassermann test has both tuberculosis and syphilis, seems entirely justifiable. We are dealing with two of the most common and most serious infectious diseases, either one of which usually lasts through a long period of years, naturally predisposing to any other infection, and therefore it is not strange that a large proportion of syphilitics should be tubercular, and perhaps the reverse is almost equally plausible, owing to the widespread distribution of syphilis.

Miller,⁸ using an antigen prepared by grinding tubercle bacilli with table salt and then adding water up to isotonicity, obtained 229 (98 per cent.) positive complement-fixation tests out of 232 clinically active cases, all of which had tubercle bacilli in the sputum. In 90 inactive or healed cases he found a negative reaction in 83 (7 were positive, and in 5 of these tubercle bacilli had recently been found in their sputum). In 140 cases in which the diagnosis was doubtful 32 gave a positive tuberculosis complement-fixation test. In 45 cases giving the positive Wassermann reaction, unlike Bronfenbrenner, he found only 2 with positive tuberculosis complement-fixation—in one of these the diagnosis of both syphilis and tuberculosis was subsequently established.

It was my intention to have this test made in my clinical laboratory with both the Wassermann and Neisser complement-fixation tests in practically every case, in order to get as broad a view-point of its clinical application as possible in both a positive and negative way. For various reasons this was not fully realized, but during the past year 135 complement-fixation tests have been made. While the number is not large the cases, for the most part, were

⁸ Jour. Lab. and Clin. Med., August, 1916, p. 816.

very carefully studied, and it seemed that their analysis, with a brief review of and comparison with other recent work and my own conclusions based thereon, might be of sufficient interest to warrant its presentation.

Craig's antigen and an antihuman amboceptor derived from the rabbit were used in all the work, and the technic employed was that of Noguchi, simply substituting one antigen for another, or using several antigens with the same serum.⁹

For reasons which appear sufficient, clinical importance was attached to smaller degrees of inhibition than with the Wassermann test. If approximately 25 per cent. of inhibition occurred, or even less with perfect hemolysis in the serum control, it was regarded as significant and followed up by further investigation, because the complement-binding antibodies, unlike those in the Wassermann test, are specific. Of these 135 cases, 36 were regarded as cases of tuberculosis upon clinical grounds. In 22 tubercle bacilli had not been found, while they had been demonstrated in 14. Of the 22 with no bacilli present, 18 (or 77 per cent.) gave a positive complement-fixation test and 4 negative, while of the 14 cases with bacilli, 10 (or 71 per cent.) gave a positive and 4 a negative result.

There were 8 cases regarded as simply suspicious. Of these, 2 gave a positive complement-fixation and 6 a negative.

Nine cases, so far as could be determined, were normal in every respect. Of these 1 was positive and 8 negative. The one case giving a positive fixation, and supposed to be perfectly healthy, developed clinical tuberculosis within two or three months, which is now progressing. One other case in this group was regarded with suspicion, but gave a negative result and remains well.

Seventy-four cases were suffering from other diseases but were without suspicion of tuberculosis. Of these, 15 gave a positive and 59 a negative complement-fixation test.

The subcutaneous tuberculin test was made 16 times in the 135 cases. It was positive 4 times and negative 12 times. The 4 cases giving a positive tuberculin reaction all gave positive complement-fixation tests. Of the 12 cases in which the subcutaneous test was negative, 9 gave a negative complement-fixation test and 2 positive; one was positive at one time and negative at another.

In regard to the very interesting question of "cross-fixation" with the Wassermann test, my results are perhaps somewhat more discouraging than Bronfenbrenner's. In 95 cases both tests were made. There were 25 positive Wassermans in the total of 95. Of these, 11 (or 44 per cent.) gave a positive tuberculosis complement-fixation test. This is 6 per cent. higher than that obtained in Bronfenbrenner's cases. In this connection it might be remarked that of the 11 cases with positive Wassermans which also gave

positive tuberculosis complement-fixation tests in 2 the clinical evidence was in favor of tuberculosis and in 9 against it. In two or three tuberculosis was plausible and in the remainder very improbable. Of the 70 with negative Wassermann, 20 gave positive tuberculosis tests or 28 per cent. as against 44 per cent. of those with a positive Wassermann. Of the cases with a positive tuberculosis complement-fixation test in which also the Wassermann was made, 31 in number, 11 (or 35 per cent.) had positive Wassermans.

The results obtained by Miller with the bacillary suspension and by Bronfenbrenner with the tuberculin type differ remarkably in the "cross-fixation" obtained in cases with a positive Wassermann. In 45 cases of Miller's giving a positive Wassermann, 2 gave a positive tuberculosis complement-fixation, while Bronfenbrenner found in 108 cases of syphilis in which tuberculosis was not suspected, 41 cases (or 38 per cent.) giving positive tuberculosis complement-fixation. On the other hand, Craig, using an antigen of the same type as Bronfenbrenner, with some difference in the technic of preparation did not get a single positive tuberculosis complement-fixation in 150 cases showing a positive Wassermann. These discrepancies are somewhat difficult to reconcile. In both Craig's and Miller's cases of syphilis the percentage of cases giving positive tuberculosis complement-fixation is smaller than the actual number of cases one would expect to find in such a group, while in Bronfenbrenner's and in my own it is undoubtedly very large.

What then is the real clinical value of the tuberculosis complement-fixation test? As indicated at the outset the real problem is not to determine the presence or absence of tubercular foci in any part of the organism but the clinical importance of such foci. We content ourselves by classifying the cases as active and inactive. Let us inquire just what we mean by these terms. Obviously the question of activity may be considered from either a pathological or clinical view-point, the latter always including the former. The case is clinically active only when it is manifested by symptoms or signs, or both. In the absence of clinical manifestations a tubercular focus may be mildly active without disturbing its host, or even entirely quiescent, but the tubercle bacilli are still viable and capable of renewed activity under favorable conditions. Between the foci which are pathologically completely quiescent and those which are producing more or less severe clinical manifestations there must be a group of "border-line" cases which might readily pass into one or the other of these classes, *i. e.*, active or inactive, and which clinical observation and the autopsy room prove to us do actually migrate back and forth perhaps many times. The criteria heretofore available for gauging the clinical status of the "border-line" case have been unsatisfactory. The presence even of tubercle bacilli in the sputum only proves that a focus which indeed may be very small and producing no constitutional

disturbance, and would be entirely unrecognizable but for these bacilli, is discharging its contents into a bronchial tube. Such a focus might or might not pour toxins into the circulation, and upon whether it does so or not would naturally depend the presence or absence in the blood of complement-binding or other tubercular antibodies. The absence of tubercle bacilli from the sputum is equally inconclusive. The same thing may be said of the subcutaneous tuberculin test. Physical signs also fail us because they are not always recognizable when most needed and they may be obtained in a healed or rapidly healing focus. In view of all these facts and the demonstrable inconstancy of tuberculosis complement-binding antibodies, what can be reasonably expected from the complement-fixation test? While I believe that this test has arrived at the stage of clinical usefulness, yet its limitations are clearly defined. The absurdity of basing a clinical diagnosis upon any single laboratory test must be obvious to anyone upon the slightest reflection. This may be unfortunate, but in spite of these limitations the clinician should accept the information which it gives at its face value and make it a part, but only a part, of the basis of his clinical judgment. Viewed in this, its proper light, my own conviction is that with either the Besredka, Craig, or Miller antigen it is probably as reliable as and much more widely applicable than the subcutaneous test without the hazards of the latter. The positive complement-fixation obtained in unselected cases with varying pathology probably represents the "border-line" cases above referred to. Bronfenbrenner found 8 per cent. of 75 such cases giving positive fixation. In my own series of 65 such cases, 13 (or 20 per cent.) gave a positive complement-fixation test. My own results in this class of cases were in remarkable contrast to those of Craig, using presumably the same antigen. My own antigen was not made by Craig, however, but in one of our large biological laboratories,¹⁰ and it may have varied slightly from Craig's formula; and then, too, it is possible that in the class of patients with which Craig had to deal, presumably men accepted for service in the United States Army because of their freedom from disease, tuberculosis may have been exceptionally rare, and, of course, by the very terms of his classification a case of suspected tuberculosis would be eliminated from this group. In spite of all this, however, it is remarkable that in 100 patients suffering from other diseases, everyone of them should have given a negative complement-fixation test. In view of all the facts, and in spite of some discrepancies, I believe, with both Bronfenbrenner and Craig, that the tuberculosis antigen will not fix the complement in the presence of syphilitic lipoid antibodies unless tubercular antibodies are also actually present. The vital question as to whether the tuberculosis complement-

¹⁰ Parke, Davis & Co.

fixation test can distinguish between clinically active and inactive cases cannot be given a positive answer excepting with the qualifications above indicated. Bronfenbrenner cautiously says that it seems to answer the requirements both of early diagnosis and of differentiation of active tuberculosis from that in which the pathological condition has apparently been arrested. This is safely conservative, but does not cover the "border-line" cases already referred to. Miller says that with his antigen inactive cases, as a rule, are negative. It seems to me that our conclusion at present must be that complement-binding antibodies are or may be present in the blood of both clinically active and inactive cases; that they may be intermittently absent in both groups of cases but more constantly present in active than in inactive cases, and that the fact of their presence as revealed by this test is valuable information which decides nothing but the existence of a focus which is at least pathologically active.

SUMMARY. Specific complement-binding bodies are present in the blood of patients suffering from tuberculosis. They are not constantly present even in clinically active cases and may be present in cases which have no clinical manifestations. Their presence may be reliably demonstrated by the usual technic. A positive result proves the existence of a tubercular focus, which is at least pathologically active. Its clinical importance, and its relationship to any coexisting syndrome, can only be decided by correlating it with all available clinical data, precisely as in the case of a positive Wassermann in an obscure case. A negative fixation test does not absolutely exclude clinically active tuberculosis any more than a single negative Wassermann excludes clinically active syphilis.

With antigens made from tuberculins or bacillary suspensions the results are at present probably as dependable as the subcutaneous tuberculin test. It is equally applicable in all cases at all times, regardless of fever, low resistance, etc. The subcutaneous tuberculin test, aside from being absolutely prohibited by universal consent in a large group of cases, has in it an element of possible danger difficult to estimate because of the possible or even probable remoteness of effect. Cross-fixations with the Wassermann test mean both tubercular and syphilitic foci. The tuberculosis complement-fixing antibodies are not lipotropic in character but are specific.

While some striking discrepancies exist in clinical reports, the main facts are unimpaired and with due regard to its obvious limitations, the tuberculosis complement-fixation test is a valuable addition to our diagnostic methods.

I wish to express my indebtedness to my clinical assistants, Dr. M. F. Porter, Jr., Dr. B. M. Edlavitch, and Dr. G. B. Kramer for their coöperation in this work.